EFFECT OF HUMIDITY ON THE PHOTOSENSITIVE RESPONSE TO 8-METHOXYPSORALEN*

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Photosensitizing effects of low molecular weight chemicals have been investigated in detail since 1898 when Raab noted the lethal action of acridine on paramecia in the presence of light (1). Blum (2) and Lipson and Baldes (3) carried out extensive studies on the biologic effects of alterations in dosage of the photosensitizer and the intensity of irradiation. Recently the effects of temperature have been studied on contact photosensitivity (4, 5) and "sunburn" erythema (6).

We are not aware of any previous studies concerning the influence of humidity on photosensitivity reactions. Accordingly, investigations were designed to assess the effects of different degrees of relative humidity on the photosensitive response to topically and systemically administered 8-methoxypsoralen (8-MOP) when other factors such as light intensity, concentration of drug and temperature were kept constant.

MATERIALS AND METHOD

Experimental Animals: Hartley strain albino guinea pigs weighing 375 gms +/− 25 gms were used. Each animal was housed in a separate cage. The diet consisted of Hemlock Hollow Farm guinea pig pellets, fresh greens twice weekly and water *ad lib*.

Temperature and Humidity: All animals received topical application of the photosensitizer as well as irradiation in a temperature and humidity controlled room. A constant temperature of 37° C. +/− 2° was maintained during application of the photosensitizer and during subsequent irradiation. A relative humidity of 30% +/− 2% was maintained for a group of 24 guinea pigs and a relative humidity of 80% +/− 2% for a second group of 17 guinea pigs. The photosensitizer, 8-MOP, was applied, and thirty minutes later the animals were irradiated for a period of 30 minutes. Following this procedure they were returned to the regular animal room where the temperature was 28° C. +/− 2° and the humidity varied from 40% to 60%.

Topical Application: The dorsal surface of the animals was depilated using a barium sulfide–zinc oxide–cornstarch mixture. Animals manifesting erythema thirty minutes following depilation were not used in the experiment. The back was divided into quadrants and 0.50 ml of an 8-MOP solution in 95% ethyl alcohol was applied to a 2 cm^2 skin surface area with a glass tipped rod. The different concentrations applied to the quadrants ranged from 3.10^{-3} M to 3.10^{-6} M. Previous tests indicated that these concentrations do not produce primary irritant reactions in non-irradiated skin.

Systemic Administration: Each of 8 guinea pigs received a 5 mg capsule of 8-MOP by mouth and was irradiated two hours later.

Light Source: Animals were irradiated in pairs from a bank of 4 Westinghouse "black" light fluorescent tubes thirty minutes after the various concentrations of 8-MOP had been applied to the quadrants. In order to exclude all radiation below 3200Å, a window glass filter, 3 mm thick, was interposed between the animals and the light source.

The experiments were carried out under the following conditions: target skin distance 25 cm; time of irradiation 30 minutes; energy-1200 microwatts/cm^2; emission spectrum 3200Å–4400Å. Control animals received only the 8-MOP solution and no light, or only light and no 8-MOP solution.

Assessment of Reactions: The test sites were observed for erythema immediately after irradiation and after 24 and 48 hours. The results at 24 and 48 hours were found to be essentially similar and were averaged on the few occasions where differences were noted. An arbitrary scale for erythema was used from zero to four as follows:

0 = no erythema
0.5 = questionable erythema
1 = minimal but definite erythema
2 = moderate erythema
3 = considerable erythema
4 = maximal response with edema

RESULTS

Topical Application.—Erythema threshold: Table I indicates that at 30% relative humidity minimal erythema was observed only at an 8-MOP concentration of 3.10^{-4} M and above; as a matter of fact only 1 of 24 animals (#18) showed an unequivocal reaction at the 3.10^{-4} M concentration. However, as indicated in Table II, at a relative humidity of 80% 16 of 17 ani-

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TABLE I

Photosensitive response to topically applied 8-MOP at 30% relative humidity

Guinea Pig #	Intensity of Erythema					
	Concentration of 8-MOP					
	3.10^{-3} M	$1.5.10^{-3}$ M	3.10^{-4} M	$1.5.10^{-4}$ M	3.10^{-5} M	3.10^{-6} M
14	1.75		0.5		0.0	0.0
15	1.50		0.0		0.0	0.0
16	2.00		0.0		0.0	0.0
17	1.75		0.0		0.0	0.0
18	2.00		1.0		0.0	0.0
19	1.50		0.0		0.0	0.0
20	2.00		0.0		0.0	0.0
21	1.75		0.0		0.0	0.0
22	2.00		0.5		0.0	0.0
23	2.25		0.0		0.0	0.0
24	2.25		0.0		0.0	0.0
25	1.75		0.0		0.0	0.0
38	1.50		0.0		0.0	0.0
39	1.50		0.0		0.0	0.0
40	1.50		0.0		0.0	0.0
41	1.75		0.0		0.0	0.0
42	1.50		0.0		0.0	0.0
43	1.50		0.0		0.0	0.0
44	1.25		0.0		0.0	0.0
45	1.50		0.0		0.0	0.0
55	1.00	0.50	0.0	0.0		
56	1.50	1.00	0.0	0.0		
57	2.00	1.50	0.0	0.0		
58	2.50	1.50	0.0	0.0		
Totals 24	41.50	4.5	2.0	0	0	0
Mean 1	1.73	0.13	0.08	0	0	0

mals showed erythema at the 3.10^{-4} M concentrations and 2 of 4 animals tested reacted at the $1.5.10^{-4}$ M concentration. The differences in erythema threshold observed are highly significant ($p < 0.01$).

Magnitude of contact photosensitive response: Erythema was seen in all 41 animals tested at a 3.10^{-3} M concentration of 8-MOP. However the magnitude of the erythema response averaged 2.2 at 80% humidity as compared to 1.8 at 30% humidity.

Systemic Administration.—Table III indicates that no significant differences in the erythema responses were observed at 30% and 80% relative humidity after systemic administration of the photosensitizer. Both the threshold and magnitude of the erythema response were essentially similar.

DISCUSSION

Contact photosensitive reactions in man and/or guinea pigs are known to be influenced by diverse factors including: 1) type and amount of light; 2) concentration and vehicle of photosensitizer; 3) temperature of environment; 4) specific skin site tested; and 5) keratin and melanin content of skin. Our studies were designed to keep the above factors constant and add one new variable, namely relative humidity. The results clearly indicate that under such standardized conditions there is a statistically significant increase in contact photosensitivity

TABLE II

Photosensitive response to topically applied 8-MOP at 80% relative humidity

Guinea Pig #	Intensity of Erythema					
	Concentration of 8-MOP					
	3.10^{-3} M	$1.5.10^{-3}$ M	3.10^{-4} M	$1.5.10^{-4}$ M	3.10^{-5} M	3.10^{-6} M
10	1.75		1.25		0.0	0.0
11	1.75		1.25		0.0	0.0
26	2.25		1.50		1.0	0.0
27	2.25		1.00		0.0	0.0
28	2.25		1.25		0.0	0.0
29	2.25		1.25		0.0	0.0
30	2.50		1.00		0.5	0.0
31	2.00		1.00		0.0	0.0
32	2.00		1.00		0.0	0.0
34	2.00		1.50		0.0	0.0
35	2.00		1.25		0.0	0.0
36	2.00		1.25		0.0	0.0
37	2.25		1.50		0.0	0.0
50	2.75	2.00	0.50	1.00		
51	2.25	2.00	1.25	0.50		
52	2.75	2.50	1.50	1.00		
53	2.25	2.00	1.75	0.0		
Totals 17	37.25	8.50	21.0	2.5	1.5	0
Mean 1	2.19	2.13	1.24	0.63	0.1	0.0

at higher relative humidity. The failure of higher relative humidity to increase the erythema response after oral administration of 8-MOP strongly suggests that the increased response after topical application is largely due to increased percutaneous absorption.

This finding does not represent an isolated phenomenon related to contact photosensitivity but is another demonstration of increased percutaneous absorption of small molecular weight compounds at higher humidity. Smith *et al.* (7) reported in 1919 that mustard gas produces more severe injuries on human skin which is "hot and sweaty" than on cool and dry skin sites. Sulzberger, Baer, Kanof and Lowenberg (8) reviewed previous published work on the effects of humidity on the effects of mustard gas and reported their own experiences which indicated that the vesicant power of lewisite is increased at higher relative humidities. Renshaw (9) and Cullumbine (10) found that water in liquid phase effectively increases skin penetration of chemical agents. Definitive studies involving water in a vapor phase as a variable were recently reported by Fritsch and Stoughton (11) using an experimental model which measured the penetration of C^{14} labelled acetylsalicylic acid through skin at various environmental conditions. Their findings demonstrated that increasing the relative humidity increases the percutaneous absorption in *excised* human skin. The exact molecular process which enables humidity to facilitate percutaneous absorption remains unknown.

The influence of relative humidity on cutaneous photosensitivity reactions potentially has considerable practical importance. It is quite possible that conditions of high ambient relative humidity significantly increase the occupational hazards associated with exposure to psoralens (12) and pitch (13) and other photosensitizers, and that these hazards can be minimized by lower humidity. Moreover, it appears likely that high degrees of relative humidity are among those factors which create suitable conditions for the occurrence of berlock dermatitis (14).

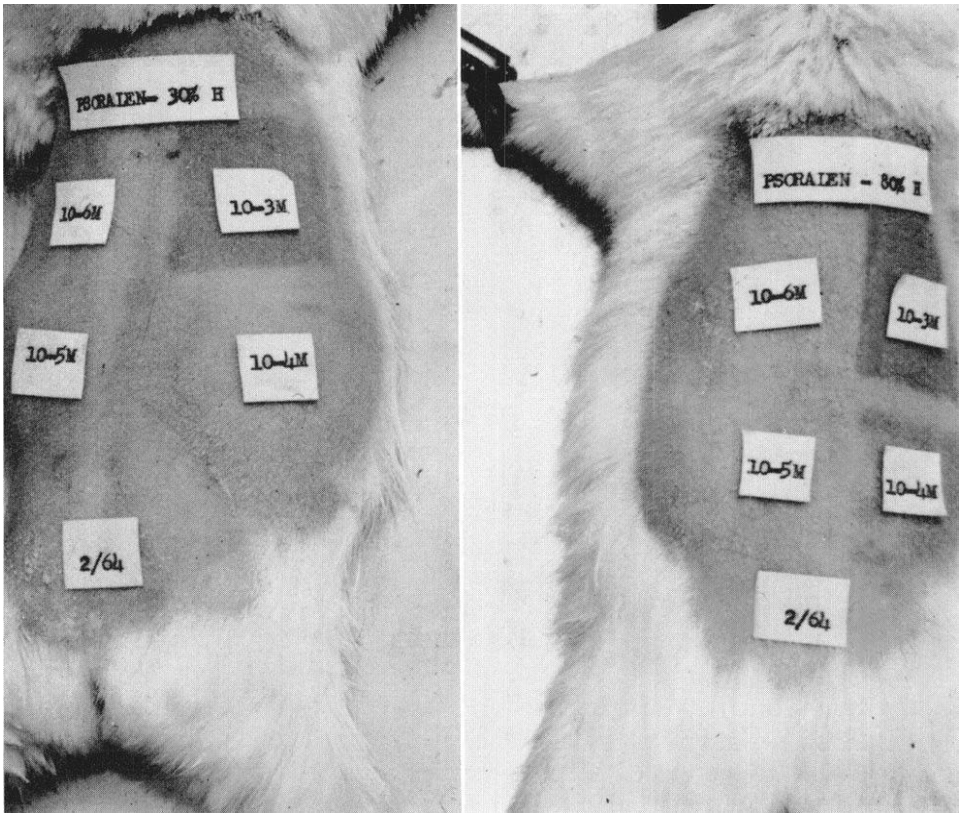

FIG. 1. Photosensitive response noted only with a 3.10^{-3} M solution of 8-MOP at 30% relative humidity (left). Photosensitive response noted with both a 3.10^{-3} M and 3.10^{-4} M solution of 8-MOP at 80% relative humidity (right).

TABLE III

Photosensitive response to orally administered 8-MOP at 30% and 80% relative humidity

Guinea Pig #	Relative Humidity	Time of Exposure			
		5 Min.	10 Min.	15 Min.	20 Min.
	%				
A	30	0	0	0	.5
B	30	0	0	1	2
C	30	0	0	1	1.5
D	30	0	0	.5	1
E	80	0	0	1	1.5
F	80	0	0	.5	1.5
G	80	0	0	0	.5
H	80	0	0	.5	1

Future investigations with these and other compounds might well indicate diverse factors such as chemical configuration, solubility, ionization and other states which determine the effects of relative humidity on percutaneous absorption.

SUMMARY

1) An increase in relative humidity from 30% to 80% significantly increased the photosensitivity response to topically applied 8-methoxypsoralen.

2) An increase in relative humidity from 30% to 80% had no effect on the photosensitivity response to systemically administered 8-methoxypsoralen.

3) The increased photosensitivity response to a topically applied photosensitizer at higher relative humidity presumably is largely due to an increase in percutaneous absorption of the photosensitizer.

4) The possible practical significance of these findings with respect to occupational and cosmetic photosensitizers is discussed.

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